SUPPLEMENT ARTICLE

Molecular Characterization and Phylogenetic Analysis of the Hemagglutinin 1 Protein of Human Influenza A Virus Subtype H1N1 Circulating in Kenya During 2007–2008

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Background. Among influenza viruses, type A viruses exhibit the greatest genetic diversity, infect the widest range of host species, and cause the vast majority of cases of severe disease in humans, including cases during the great pandemics. The hemagglutinin 1 (HA1) domain of the HA protein contains the highest concentration of epitopes and, correspondingly, experiences the most intense positive selection pressure.

Objectives. We sought to isolate and genetically characterize influenza A virus subtype H1N1 (A[H1N1]) circulating in Kenya during 2007–2008, using the HA1 protein.

Methods. Nasopharyngeal swab specimens were collected from patients aged ≥2 months who presented to 8 healthcare facilities in Kenya with influenza-like illness. We tested specimens for seasonal influenza A viruses, using real-time reverse-transcription polymerase chain reaction (RT-PCR). Viruses were subtyped using subtype-specific primers. Specimens positive for seasonal A(H1N1) were inoculated onto Madin-Darby canine kidney cells for virus isolation. Viral RNAs were extracted from isolates, and the HA1 gene was amplified by RT-PCR, followed by nucleotide sequencing. Nucleotide sequences were assembled using BioEdit and translated into amino acid codes, using DS Gene, version 1.5. Multiple sequence alignments were performed using MUSCLE, version 3.6, and phylogenetic analysis was performed using MrBayes software.

Results. We found that, similar to A/Brisbane/59/2007 (H1N1)–like virus, which was included in the southern hemisphere vaccine for the 2009 influenza season, all 2007 Kenyan viruses had D39N, R77K, T132V, K149R, and E277K amino acid substitutions, compared with A/Solomon Islands/3/2006 (H1N1)–like virus, a component of the southern hemisphere vaccine for the 2008 influenza season. However, the majority of 2008 viruses from Kenya also had R192K and R226Q substitutions, compared with A/Solomon Islands/3/2006 (H1N1)–like virus. These 2 changes occurred at the receptor binding site. The majority of the 2008 Kenyan isolates contained N187S, G189N, and A193T mutations, which differed from A/Brisbane/59/2007 (H1N1)–like virus. The A193T substitution is involved in binding the sialic acid receptor. Phylogenetically, the 2008 Kenyan isolates grouped into 2 clusters. The main cluster contained viruses with N187S and A193T changes; residue 187 is involved in receptor binding, whereas residue 193 is at antigenic site Sb.

Conclusion. Overall, the major genetic variations that occurred in seasonal A(H1) viruses either affected receptor binding or altered epitopes at the immunodominant sites. These genetic variations in seasonal A(H1N1) isolated in Kenya during 2007–2008 highlight the importance of continuing surveillance and characterization of emerging drift variants of influenza virus in Africa.

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Among influenza viruses, type A viruses cause the vast majority of severe disease in humans, including pandemics. They also infect the widest range of host species and exhibit the greatest genetic diversity [1]. The genetic diversity of influenza viruses is caused by the error-prone RNA polymerase during genome replication [2, 3]. The error-prone replication and short generation times result in large, highly diverse replicating populations of viral quasi-species upon which natural selection operates [4]. Investigating the complex factors that affect this natural selection is key to understanding influenza A virus evolution. Modern molecular and phylogenetic analysis tools are often applied to understand these processes [5, 6].

While both hemagglutinin (HA) and neuraminidase (NA) proteins contain immunogenic sites at which immune-driven natural selection happens, the N-terminal (HA1) domain of the HA protein contains the maximum concentration of epitopes and experiences the strongest positive selection pressure [7]. The majority of neutralizing antibodies are directed toward 5 distinct immunogenic sites on the HA1 globular domain: Sa, Sb, Ca1, Ca2, and Cb [8, 9]. Each of these immunogenic sites is a conformational entity formed by a number of amino acid residues folded in 3-dimensional space [8, 9]. Any change occurring at these immunogenic sites will affect the ability of preexisting antibodies to neutralize the virus. Because the human immune response to viral infection is incompletely cross-protective, amino acid variants of the HA1 protein that allow the virus to evade immune response to infect more hosts are favored by natural selection processes [7]. In addition to the central role of the HA1 domain as an immune response determinant, it is also the influenza virus ligand containing the binding site for attachment to the host cell receptors [10, 11]. The high affinity of HA1 for human-like α(2,6) receptors and the significant difference in sequence and immunogenicity of HA1 among existing seasonal and vaccine strains are the hallmark pathogenicity determinants for influenza viruses, influencing the ease of human-to-human transmission. Within HA1, D190, and D225 residues are key determinants for effective binding to human-like α(2,6) receptors [10, 12, 13]. A single mutation, D225G, reduces HA's binding affinity for α(2,6) receptors [12, 13] and the infectivity of the virus [10], while a double variant, D190E/D225G, eliminates HA's binding affinity to α(2,6) receptors [12, 13] and renders the virus noninfectious [10].

Kenya is an East African country that straddles the equator and has a tropical climate. Although 2 reports showing that influenza A virus subtype H3N2 and influenza B virus circulated in Kenya between 2004 and 2006 [14, 15], there has not been documentation of the presence and molecular characterization of influenza A virus subtype H1N1 (A[H1N1]) in Kenya. This is mainly due to a lack of influenza surveillance in the country [14, 15]. Although influenza vaccine is rarely used in Kenya, the Kenya Ministry of Health recommends the use of the southern hemisphere influenza vaccine formulation for its citizens and other residents.

In 2006, in collaboration with the US Centers for Disease Control and Prevention (CDC) and the Kenya Ministry of Public Health and Sanitation, the US Army Medical Research Unit established syndromic and virologic surveillance for influenza at sentinel health facilities throughout Kenya [14]. From respiratory specimens collected through this surveillance system during 2007–2008, we isolated and analyzed A(H1N1). By using the nucleotide sequences of the globular HA1 region of the HA gene, we provide the first description of the molecular and phylogenetic characteristics of A(H1N1) circulating in Kenya.

MATERIALS AND METHODS

Setting
We conducted surveillance for patients with influenza-like illness (ILI) at 1 provincial hospital in Kisumu city in western Kenya and in 7 district hospitals in the cities of Nairobi, Mombasa, Malindi, Isiolo, Kericho, Kisii, and Alupe. Surveillance hospitals were chosen because they were geographically and demographically representative of the population of Kenya.

Study Population
Patients with ILI included anyone >2 months of age presenting to a surveillance hospital outpatient clinic with fever and either cough or sore throat. We excluded presentations of ILI in patients with exudative pharyngitis or tonsillitis or symptom onset >72 hours prior to presentation. Surveillance officers enrolled the first 5 case patients with ILI presenting to each surveillance hospital daily and obtained informed consent from patients for study participation. The Kenya Medical Research Institute (KEMRI), the Walter Reed Army Institute of Research (WRAIR), and the CDC institutional review boards reviewed and approved the study protocol under protocol numbers SSC 981, WRAIR 1267, and CDC 4716, respectively.

Specimen Collection
Surveillance officers collected duplicate nasopharyngeal samples from each case patient, using Dacron-flocked swabs. Swabs were placed in a 1-mL cryovial containing virus transport medium, kept at 4°C, and stored in a liquid nitrogen dry shipper within 8 hours of collection. All samples were transported from the surveillance sites to the Kenya National Influenza Center (NIC) laboratory within 1 week. The cold chain was maintained throughout.

Laboratory Testing
Real-Time Reverse-Transcription Polymerase Chain Reaction (RT-PCR)
For detection of influenza virus in patient samples, total RNA was extracted from the samples, using the QIAamp Viral RNA

Mini Kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. One-step real-time PCR was performed on an ABI 7500 Fast platform, according to the CDC protocol for the identification of A(H1) viruses. The total reaction volumes were 25 μL, containing 0.5 μL of superscript III/Platinum enzyme mix (Invitrogen, Paisley, United Kingdom), 5.5 μL of H_2O, 12.5 μL of 2X buffer, 0.8 μM forward primer (40 μM), 0.8 μM reverse primer (40 μM), and 0.2 μM probe (10 μM). Each RNA sample was tested for 6 sets of gene targets: matrix gene segment, to identify influenza A and B viruses; HA gene segment, to subtype H1, H3, and H5 viruses; and human RNP gene segment, to test the RNA extraction procedure. Reverse transcription was achieved at 50°C for 30 minutes and 95°C. PCR was achieved after 45 cycles of 95°C for 15 seconds and 55°C for 30 seconds.

Virus Isolation

Influenza virus isolation was performed at the NIC in Madin-Darby canine kidney cells. Afterward, we conducted a hemagglutination inhibition (HAI) assay, using guinea pig red blood cells and reference antiserum in accordance with CDC protocols. Inoculated cells were incubated at 37°C with 5% CO_2 and observed daily for 10 days for visual cytopathic effect, using an inverted microscope. When the cytopathic effect was observed, the supernatant fluid was collected, and the hemagglutination titer was measured. HAI testing was performed on high-titer (≥32:1) samples. Isolates with a low hemagglutination titer (<32:1) were repassaged once. On the basis of the variability in HAI titers, we selected 46 isolates for further molecular genotyping, and these were confirmed as A(H1N1). The guinea pigs used to provide blood for HA and HAI assays of this report were reared in accordance with KEMRI guidelines on animal care.

PCR Assay and Nucleotide Sequencing of the HA1 Gene Segment

A(H1N1) isolates were sent to US Air Force School of Aerospace Medicine (USAFSAM; San Antonio, TX), where they were processed for genome sequencing. The USAFSAM served as the confirmatory laboratory for HAI tests done at KEMRI. Isolates were molecularly typed and subtyped at the USAFSAM, and results were forwarded to the CDC in Atlanta, a WHO Influenza Collaborating Centre.

RNA was extracted from 48-hour shell vial cultures at the USAFSAM, using the M48 BioRobot and Mag Attract Virus Mini M48 Kit (Qiagen) according to the manufacturer's protocols. For RT-PCR amplification, 5 μL of RNA was added to a 50-μL master mixture containing 2 × reaction buffer, 1 × enzyme mixture, and 400 nmol/L each of CDC primers (H1HA_26_F_M13, 5′-TGT AAA ACG ACG GCC AGT CAA CCA AAA TGA AAG-3′; and H1HA_1238_R_M13, 5′-CAG

GAA ACA GCT ATG ACC AGC TGT GAA TTG RGT GTT CAT TT-3′), using the SuperScript III One-Step RT-PCR System (Invitrogen, Carlsbad, CA). RT-PCR thermocycling consisted of cycles at 50°C for 30 minutes, hot start activation at 94°C for 2 minutes, 40 amplification cycles at 94°C for 15 seconds, at 52°C for 30 seconds, and at 68°C for 75 seconds, with a final extension cycle at 68°C for 5 minutes. All PCR products were visualized as 1200–base pair bands after gel electrophoresis in 2% precast agarose gels stained with ethidium bromide (Invitrogen) under UV illumination. PCR products were purified using the QIAquick PCR Purification Kit (Qiagen). Incorporation of fluorescent nucleotides was facilitated using the Big Dye Terminator v3.1 Kit (Applied Biosystems, Foster City, CA) and sequenced using terminal H1HA_26_F_M13 and H1HA_1238_R_M13 RT-PCR primers and 6 internal oligonucleotides: H1HA_522_R_M13 (5′-CAG GAA ACA GCT ATG ACC CGT CAG CCA TAR CAA ATT T-3′), H1HA_239_F_M13 (5′-TGT AAA ACG ACG GCC AGT TGG GTA AYT GCA GCR TTG C-3′), H1HA_760_R_M13 (5′-CAG GAA ACA GCT ATG ACC GTC AGT AGT AR TTR ATT CT-3′), H1HA_F3_487 (5′-TGT AAA ACG ACG GCC AGT AAT TTG CYA TGG CTG ACG-3′), H1HA_1063_R_M13 (5′-CAG GAA ACA GCT ATG ACC GCA ATG GCT CCA AAC AAA CCT CT-3′), and H1HA_F4_682 (5′-TGT AAA ACG ACG GCC AGT CCA GAA ATA RCC AAA AGA C-3′).

HA1 Nucleotide Genetic Analyses

Nucleotide contigs were assembled using the Contig Assembly program in Bioedit [16]. Protein translations were performed using DS Gene, version 1.5 (Accelrys, Cambridge, United Kingdom). Multiple sequence alignments were performed with MUSCLE, version 3.6 [17]. Phylogenetic analyses were performed with MrBayes 3.1 [18]. The generalized time-reversible model parameters and priors were incorporated into the nexus file for execution in MrBayes. The data were executed in MrBayes by running 1 million Monte Carlo Markov chains with a sampling frequency of 1000. The phylogenetic tree was visualized using FigTree, version 1.3.1 [19]. The protein sequences for the Kenyan A(H1N1) isolates are available from GenBank (accession numbers FJ662910.1–FJ662927.1, FJ265654.1–FJ265662.1, and EU204969.1–EU770350.1).

The nucleotide and deduced amino acid sequences of HA1 from isolates were compared with those of vaccine strains, as well as with those of strains from another African country (South Africa) and 2 other tropical countries (Malaysia and Thailand). Thus, the HA1 of A/Brisbane/59/2007 (H1N1)–like virus (the recommended southern hemisphere vaccine strain for the 2009 influenza season), A/Solomon Islands/3/2006 (H1N1)–like virus (the recommended southern hemisphere vaccine strain for the 2008 season), and A/New Caledonia/20/99(H1N1)-like virus (the recommended southern hemisphere

vaccine strain for the 2000–2007 seasons) were included in the analysis.

RESULTS

From January 2007 through November 2008, we enrolled and collected nasopharyngeal swab specimens from 5898 children and adults who received a diagnosis of ILI. The median age of enrolled case patients was 18 months (range, 2 months–75 years), and 5629 specimens (94.5%) were from children. None of the patients sampled had previously been vaccinated against influenza.

All samples tested by real-time RT-PCR were positive for the human RNP housekeeping gene, but only 1177 samples were positive for the matrix gene of influenza A virus. Of the 1177 matrix gene–positive samples, 72 representative isolates (6.1%) for each month of collection and from diverse geographical regions of the country were randomly selected for further testing. On the basis of variability in HAI titers, 46 of 72 isolates (65%) were confirmed as A(H1N1) and were selected for further molecular genotyping.

Compared with the reference influenza vaccine strains and the influenza strains from South Africa and tropical countries (Malaysia and Thailand), all 2007 Kenyan A(H1N1) isolates were most similar (98.6%–100%, based on nucleotides; and 98.1%–100%, based on amino acids) to A/Brisbane/59/2007 (H1N1)–like virus, followed by A/Solomon Islands/3/2006 (H1N1)–like virus (96.7%–99.9%, based on nucleotides; and 96.5%–100%, based on amino acids), and A/New Caledonia/20/99(H1N1)-like virus (95.9%–99.9%, based on nucleotides; and 98.1%–99.7%, based on amino acids).
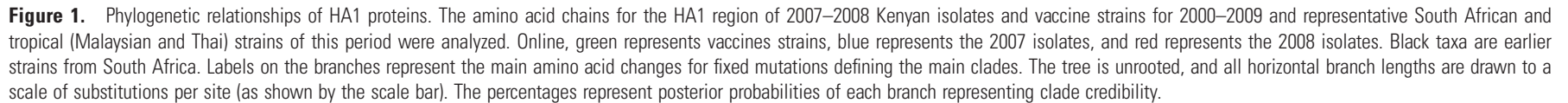
Mutational analyses of the HA1 protein that show evolution of the 2007–2008 Kenyan isolates is presented in Table 1. Compared with A/Solomon Island/3/2006 (H1N1)–like virus, all 2007–2008 Kenyan isolates had D39N, R77K, T132V, K149R, and E277K substitutions. In addition, 42% of the 2007 isolates had the Q108H change, while 31.5% of the isolates from the same year had the S125N mutation. We observed that 2 of the 2007 isolates had D225G and that all of the 2008 isolates had R226Q changes. Furthermore, of the 2008 isolates, 6 (21%), 26 (93%), and 8 (29%) had N187S, R192,K and A193T changes respectively, and 24 (86%) of the 2008 Kenyan isolates had a change affecting residue 189. Of these, 23 (96%) were G189N, and 1 isolate had a G189A mutation.

All of the 2007 Kenyan isolates had the Q108H, S125N/S125R, and D225G changes in exactly the same proportions as A/Solomon Islands/3/2006 (H1N1)–like virus. Q108H and S125N are parallel mutations found in Kenyan isolates, but have not been seen anywhere else globally, during and after the study period. In addition, 7 (37%) of the 2007 Kenyan isolates had a mutation at residue 190. The majority (6 [86%]) of these had a D190N change, and only a single isolate had the previously reported D190G mutation. The majority (24 [86%]) of the 2008 Kenyan viruses had a mutation at residue 189; 23 (96%) were G189N and the remaining had a G189A mutation. Furthermore, we discovered that of the 2008 Kenyan isolates, 6 (21%) and 8 (29%) had N187S and A193T mutations, respectively.

Table 1. Major Amino Acid Changes Present within the HA1 Protein in 2007–2008 Kenyan Influenza A (H1N1) Isolates Compared to A/Solomon Islands/3/2006 and A/Brisbane/59/2007 Vaccine Strains

Vaccine Strain	Year	
	2007	2008
A/Solomon Islands/3/2006	D39N (19/19)	D39N (28/28)
	R77K (19/19)	R77K (28/28)
	Q108H (8/19)	T132V (28/28)
	S125N (6/19); S121R (1/19)	K149R (28/28)
	T132V (19/19)	N187S (6/28) [Sb]
	K149R (19/19)	G189N (23/28); G189A (1/28) [Sb]
	D225G (2/19) [Ca2; SA]	R192K (26/28) [SA, Sb]
	E277K (19/19)	A193T (8/28) [SA, Sb]
		R226Q (28/28) [SA]
		E277K (28/28)
A/Brisbane/59/2007	Q108H (8/19)	N187S (6/28) [Sb]
	S125N (6/19); S125R (1/19)	G189N (23/28); G189A (1/28) [Sb]
	D190N (6/19); D190G (1/19) [Sb, SA]	K192R (2/28) [Sb, SA]
	D225G (2/19) [Ca2, SA]	A193T (8/28) [SA, Sb]

The numbers in parentheses are proportions of the isolates with the indicated change. Ca2, Sb, and SA denote occurrence at antigenic sites Ca2 and Sb and binding to sialic acid receptor, respectively.

Figure 1. Phylogenetic relationships of HA1 proteins. The amino acid chains for the HA1 region of 2007–2008 Kenyan isolates and vaccine strains for 2000–2009 and representative South African and tropical (Malaysian and Thai) strains of this period were analyzed. Online, green represents vaccines strains, blue represents the 2007 isolates, and red represents the 2008 isolates. Black taxa are earlier strains from South Africa. Labels on the branches represent the main amino acid changes for fixed mutations defining the main clades. The tree is unrooted, and all horizontal branch lengths are drawn to a scale of substitutions per site (as shown by the scale bar). The percentages represent posterior probabilities of each branch representing clade credibility.

A phylogenetic comparison of the 2007–2008 Kenyan isolates to the 2008 and 2009 vaccine strains and the contemporaneously circulating South African, Malaysian, and Thai isolates is presented in Figure 1. All 2007 Kenyan isolates clustered in a single clade together with A/Brisbane/59/2007 (H1N1)–like virus but away from A/Solomon Islands/3/2006 (H1N1)–like virus. Within this clade, many of the Kenyan viruses formed a subbranch away from A/Brisbane/59/2007 (H1N1)–like virus, characterized by Q108H and S125N changes. A majority (82%) of the 2008 Kenyan isolates had a G189N mutation, which was not present in A/Brisbane/59/2007 (H1N1)–like virus, and formed a clade distinct from the 2007 Kenyan isolates. This clade bifurcated further into 2 branches: the first contained viruses without the A193T change, and the second contained viruses that had this mutation. The majority of the viruses present in the A193T branch had the N187S mutation. All Kenyan viruses on the N187S branch were derived from a single sentinel site, and only viruses isolated from Johannesburg in 2008 were also present on this branch.

DISCUSSION

We have documented the evolution of A(H1N1) viruses in Kenya during a 23-month period in 2007–2008. Compared with the 2 vaccine strains, A/Solomon Island/3/2006 (H1N1)–like virus and A/Brisbane/59/2007 (H1N1)–like virus, the 2007–2008 Kenyan viruses had HA1 protein mutations at 5 important ligand interacting positions: 190, 192, 193, 225, and 226. These residues are involved in binding the human-like $\alpha(2,6)$ ligand [10, 12, 13]. The mutations detected include D190N/G, R192K $\leftrightarrow$K192R, A193T, D225G, and R226Q. Amino acids at position 190, 192, and 193 contact the extension region of $\alpha(2,6)$ ligand, and the residue at 226 interacts with the base region of the ligand [12]. A single D225G mutation reduces binding affinity for $\alpha(2,6)$ receptors [12, 13] and lowers the infectivity of the virus [10]. Double D190E/D225G mutations are lethal for the virus as they abrogate binding to $\alpha(2,6)$ receptors [12, 13], making the virus noninfectious [10]. Furthermore, mutations occurring at positions 190, 225, and 226 were present at the Ca2 antigenic site.

Overall, the ligand-interacting mutations we observed in Kenyan viruses are cumulatively deleterious to the virus and individually represent viruses evolving to less fit variants. Considering the relatively large numbers of such viruses we observed among the Kenyan isolates, A(H1N1) viruses seem to be evolving at high rates in Kenya, leading to this disproportionate level of quasi-species among the isolates.

We further sought to investigate in silico the effects that mutations occurring in the HA1 protein would have on the immunogenicity of the viruses. We found 5 mutations—D39N, R77K, T132V, K149R, and E277K—that were common to all viruses circulating in Kenya during the study period. None of

these mutations was present at an antigenic or receptor binding site. Similarly mutations at positions 192 and 193 were present at the Sb antigenic site [8, 9]. In addition, we observed that the majority of the 2008 Kenyan viruses had changes at positions 187 and 189, both located at antigenic site Sb. The G189N change found in 82% of the 2008 viruses is a major change involving replacement of the tiny nonpolar glycine residue with the charged bulkier asparagine. Because of steric and electrostatic interactions, this change is expected to alter this epitope to abrogate binding of any preexisting antibodies to the Sb site. In general, the most commonly mutated antigenic site among the 2007–2008 Kenyan viruses was the Sb site. The apparent positive selection on the Sb antigenic site is consistent with previous reports that have shown that this site is under much higher pressure for mutations than the others [9].

All 2008 Kenyan isolates had changes at the receptor binding site and multiple mutations affecting antigenic sites and were genetically distinct as compared to A/Solomon Island/3/2006 (H1N1)–like virus. Similar changes were seen globally and led to the WHO to recommend changing the vaccine A(H1) strain for 2009 to A Brisbane/59/2007 (H1N1)–like virus. Overall, our data show that the majority of the 2007 Kenyan isolates did not evolve to become genetically different from the vaccine strain and that the southern hemisphere vaccine was therefore well matched to viruses circulating in Kenya in 2007.

The clustering on the same phylogenetic branch as A/Brisbane/59/2007 (H1N1)–like virus for all the Kenyan 2007 isolates, in contrast to the single Malaysian 2007 isolate, which clustered on the same branch as A/Solomon Islands/3/2006 (H1N1)–like virus, suggested that the Kenyan 2007 isolates were closely related to viruses circulating in other parts of the world in 2007. On the other hand, clustering of the 2008 Kenyan isolates into 2 branches characterized by N187S and A193T changes and on a separate branch from A/Brisbane/59/2007 (H1N1)–like virus showed that these viruses were genetically variant from the A(H1) component of the 2009 southern hemisphere vaccine. These observations indicate that the viruses that circulated in Kenya in 2008 drifted away from the vaccine strain and that the different clusters could be due to different introduction of viruses in the country.

Although genetic analysis of the HA1 gene is important in informing our understanding of influenza virus evolution and antigenic changes, the lack of hemagglutination inhibition (HAI) data for the Kenyan viruses is a major limitation in this study. Furthermore, although HA is the principal antigen that elicits host immune response and is responsible for binding to sialic acid receptors thus mediating viral entry into host cells [20], HA is not the only protein against which antibodies are directed. Other proteins, including NA, play a role in overall host humoral response to the virus. However, antibodies to NA do not neutralize the virus as do HA antibodies [21].

Thus, analyses of HA1 genomic data alone are routinely applied to provide preliminary understanding of influenza viruses [22–24]. Another limitation of this study is the sequencing of isolates instead of viruses from clinical specimens. This may have introduced mutations not found in the original clinical samples.

Our study is the first to describe the genetic variations of A(H1N1) found in Kenya. The findings regarding changes at the receptor binding sites suggest that in Kenya, A(H1N1) is undergoing directional evolution in the HA protein as has been observed elsewhere[25]. These findings support the change of the vaccine strain in 2009 from A/Solomon Island/3/2006 to A/Brisbane/59/2007 and show that, despite minimal influenza vaccine use in Kenya, both vaccine strain choices were appropriate for Kenya. This underscores the need to promote the southern hemisphere influenza vaccine uptake in Kenya.

In conclusion, molecular analyses of circulating A(H1N1) in Kenya during 2007–2008 indicate that the viruses evolved by point mutational drift from the vaccine strains. These findings are similar to those from our earlier study of influenza A virus subtype H3N2 during the 2006–2007 season [14]. Discovery of G189N and parallel mutations Q108H and S125N unique to Kenya among A(H1N1) isolated in 2007–2008 highlights the importance of continuing surveillance and characterization of emerging influenza virus drift variants in Africa.

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